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Association of Reported Prostate Cancer Risk Alleles With PSA Levels Among Men Without a Diagnosis of Prostate Cancer

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Abstract

BACKGROUND—Prostate specific antigen (PSA) is widely used for prostate cancer screening but its levels are influenced by many non cancer-related factors. The goal of the study is to estimate the effect of genetic variants on PSA levels.

METHODS—We evaluated the association of SNPs that were reported to be associated with prostate cancer risk in recent genome-wide association studies with plasma PSA levels in a Swedish study population, including 1,722 control subjects without a diagnosis of prostate cancer.

RESULTS—Of the 16 SNPs analyzed in control subjects, significant associations with PSA levels ($P < 0.05$) were found for six SNPs. These six SNPs had a cumulative effect on PSA levels; the mean PSA levels in men were almost twofold increased across increasing quintile of number of PSA associated alleles, P -trend = 3.4×10^{-14} . In this Swedish study population risk allele frequencies were similar among T1c case patients (cancer detected by elevated PSA levels alone) as compared to T2 and above prostate cancer case patients.

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CONCLUSIONS—Results from this study may have two important clinical implications. The cumulative effect of six SNPs on PSA levels suggests genetic-specific PSA cutoff values may be used to improve the discriminatory performance of this test for prostate cancer; and the dual associations of these SNPs with PSA levels and prostate cancer risk raise a concern that some of reported prostate cancer risk-associated SNPs may be confounded by the prevalent use of PSA screening.

Keywords

genetic; bias; *KLK3*

INTRODUCTION

Prostate specific antigen (PSA) is an androgen-regulated serine protease of the kallikrein family produced by secretory epithelial cells of the prostate [1,2]. It is the most widely biomarker used for prostate cancer screening and for monitoring response to treatment. However, the predictive performance of PSA for prostate cancer is not ideal. Results from the large Prostate Cancer Prevention Trial (PCPT) showed that 25–30% of men with abnormal PSA levels (≥ 4.0 ng/ml) had cancer on prostate biopsy while ~15% of men whose PSA levels were considered to be normal, that is, <4.0 ng/ml, also had prostate cancer on biopsy [3]. The less than optimal predictive performance of PSA as a marker of prostate cancer is largely due to the fact that PSA is not prostate cancer specific. Besides the presence of prostate cancer, PSA levels are also influenced by age, prostate size, ethnicity, and the presence of inflammation/prostatitis. In addition, genetic variants in several genes such as the *PSA* gene itself (*KLK3*) and androgen receptor (*AR*) genes have been reported to be associated with PSA levels among men without prostate cancer [4–18]. However, the genetic associations between sequence variants of these two genes and PSA levels have not been consistent among published studies.

Recently, several sequence variants in the genome have been reported to be associated with prostate cancer risk in genome-wide association studies among prostate cancer cases and controls [19–26]. In some of these studies, a large proportion of prostate cancer cases were diagnosed because of elevated PSA while controls were men with low PSA levels. Therefore, the cases and controls differ in PSA levels, and consequently, identified prostate cancer risk variants may also be associated with PSA levels in controls. For example, a reported prostate cancer risk variant in the 3' of the *KLK3* gene at 19q13 (rs2735839) was found to be significantly associated with serum PSA levels among 1,646 control subjects in stage 2 of the Eeles [26] study ($P=6.1 \times 10^{-8}$).

Associations of sequence variants with PSA levels have potentially important clinical implications. If inherited variants and PSA are independently associated with prostate cancer risk, adjustment for genetic variants when interpreting PSA may result in an improvement of the predictive performance in prostate cancer detection. On the other hand, there is a concern that alleles that are associated with higher PSA levels may be over-represented in cases (detection bias) and under-representative in controls (selection bias) in populations where PSA screening is commonly performed. This PSA detection bias and selection bias may lead to false discover of prostate cancer associations.

To this end, we systematically assessed the association of prostate cancer risk variants discovered in recent genome-wide association studies. We assessed their associations, individually, and cumulatively, with PSA levels among men without a prostate cancer diagnosis.

MATERIALS AND METHODS

Study Population

The study sample was described in detail elsewhere [27]. In brief, we conducted a large-scale population-based case-control study in Sweden, named CAPS (CANCER Prostate in Sweden). Prostate cancer patients were identified and recruited from four of the six regional cancer registries in Sweden and the National Prostate Cancer Register [28]. The inclusion criterion for case subjects was histologically or cytologically verified prostate cancer, diagnosed between July 2001 and October 2003. Among 3,648 identified prostate cancer case subjects, 3,161 agreed to participate. DNA samples from blood and TNM stage, Gleason grade (biopsy), and plasma PSA levels at time of diagnosis were available for 2,893 patients. Control subjects were recruited concurrently with case subjects. They were randomly selected from the Swedish Population Registry, without a diagnosis of prostate cancer, and matched according to the expected age distribution of cases (groups of 5-year intervals) and geographic region. A total of 3,153 controls were invited and 2,149 agreed to participate. DNA samples from blood were available for 1,722 control subjects. Plasma PSA level was measured for all control subjects but was not used as an exclusion variable. A history of prostate cancer among first-degree relatives was obtained from a questionnaire for both cases and controls.

The study was approved by the research ethical committees of the Karolinska Institute, Umeå University, and Wake Forest University School of Medicine.

PSA Measurements

The levels of total PSA (tPSA) in EDTA anticoagulated plasma stored frozen at -80°C for no more than 5 years were measured by Dr. Lilja's laboratory at the Wallenberg Research Laboratories, Department of Laboratory Medicine, Lund University, University Hospital UMAS in Malmö, Sweden, using the DELFIA Prostatus® PSA-Assay (Perkin-Elmer, Turku, Finland). The lower limit of detection for tPSA is 0.05 ng/ml, the coefficient of variation (CV) is 13.9% at low (0.34 ng/ml), 5.6% at medium (2.3 ng/ml), and 5.5% at high (20.6 ng/ml) levels, and the assay results are consistent with WHO calibration standards.

Selection of SNPs for Evaluation and SNP Genotyping

We selected 16 SNPs implicated in four genome-wide association studies and replicated in at least one independent study population in the original articles (19–26). These included three SNPs at 8q and one SNP each at 2q15, 3p12, 6q25, 7p15, 7q21, 9q33, 10q11, 10q26, 11q13, 17q12, 17q24, 19q13, and Xp11.

These 16 SNPs were genotyped among case patients and control subjects using a MassARRAY QGE iPLEX system (Sequenom, Inc., San Diego, CA). Polymerase chain reaction (PCR) and extension primers for these SNPs were designed using MassARRAY Assay Design 3.0 software (Sequenom, Inc). The primer information is available at <http://www.wfubmc.edu/genomics>. PCR and extension reactions were performed according to the manufacturer's instructions, and extension product sizes were determined by mass spectrometry using the Sequenom iPLEX system. Duplicate test samples and two water samples (PCR negative controls) that were blinded to the technician were included in each 96-well plate. The average genotype call rate for these SNPs was 98.3% and the average concordance rate was 99.8%.

Statistical Analyses

We tested for Hardy–Weinberg equilibrium for each SNP among control subjects using Fisher's exact test. In all analyses, PSA levels were logarithm-transformed to best

approximate the assumption of normality. We tested the main effect of prostate cancer risk-associated alleles of each SNP on PSA levels assuming an additive model and adjusted for age and geographic region using a multiple regression model. SNPs that were associated with PSA levels ($P < 0.05$) were subsequently included in a multivariate analysis in order to test for their independent effect on PSA levels from other genetic variants, age, and geographic regions. We also estimated the proportion of variance (R^2) in PSA levels explained by age or SNPs, respectively, by fitting a linear regression model for age alone or genetic variants alone. In addition, we tested the cumulative effect of genetic variants on PSA levels for a subset of SNPs that had an independent effect on PSA levels. Subjects were clustered into five approximately equal sizes of groups (quintiles) based on the number of PSA associated alleles of SNPs (theoretically one individual can carry 0 up to 12 risk alleles of the 6 associated SNPs). A trend test was performed for the quintile (coded as 1–5, as an ordinal variable) and adjusted for age and geographic region using a linear regression model.

Allele frequency differences between subgroups of case patients were tested for each SNP using a chi-square test with 1 degree of freedom.

RESULTS

We evaluated 16 SNPs—associated with prostate cancer risk in recent genome-wide association studies—among 1,722 men without a prostate cancer diagnosis for their association with PSA levels [19–26]. Each of the 16 SNPs were in Hardy–Weinberg equilibrium. Mean plasma PSA levels (least square mean) by genotype for each SNP adjusted for age and geographic region are presented in Table I. Except for the SNPs of rs721048 at 2p15, rs9364554 at 6q25, and rs1447295 at region 1 of 8q24, men who carry either one or two copies of the reported prostate cancer risk genotypes (NR or RR) of the remaining 13 SNPs have higher mean PSA levels than those who were homozygous carriers of non-risk alleles (NN). A statistically significant ($P < 0.05$) additive effect of reported risk alleles on PSA levels was found for six SNPs, located at 7p15, 10q11, 10q26, 17q12, 19q13, and Xp11. Among them, three SNPs (rs10486567 in the JAZF1 gene at 7p15, rs10993994 in the MSMB gene at 10q11, and rs4430796 in the TCF2 gene at 17q12) were highly significant (nominal $P < 0.003$) and remained significant at a 5% study-wise error rate after adjusting for 16 independent tests using a Bonferroni correction.

The independent effect of these SNPs on PSA levels was examined by including all of the six significant SNPs (with nominal $P < 0.05$) as well as age and geographic regions in a multivariate linear regression analysis. These six SNPs and age remained significant (nominal $P < 0.05$) after adjusting for other variables in the model, suggesting their independent effect on PSA levels (Table II). The proportion of variance in PSA levels accounted for by these variables was estimated from the R^2 of the regression models; 3% was accounted for by these six genetic variants when only these SNPs were included in the model, and 8% was accounted for by age (year) when age alone was included in the model.

We also examined the cumulative effect of these PSA-associated SNPs on PSA levels. When subjects were clustered into five approximately equal-sized groups (quintiles) based on the number of PSA-associated alleles of these six SNPs, the mean PSA levels of these groups increased gradually with each increase in quintile. The trend was highly significant ($P = 3.4 \times 10^{-14}$) and the cumulative effect was independent of age. The age- and geographic-adjusted mean PSA level was 1.15, 1.48, 1.51, 1.82, and 2.01 ng/ml, respectively for the 1st, 2nd, 3rd, 4th, and 5th quintile (Fig. 1). A similar trend was found when the analysis was limited to men with younger age (< 65 years), $P = 1.1 \times 10^{-5}$, or older age (≥ 65 years), $P = 5.3 \times 10^{-10}$.

To gain insight about the effect of PSA associations of these SNPs on their observed associations with prostate cancer risk, we estimated frequencies of these alleles in subgroups of case patients. We divided cases into groups of T1c tumors (N=958), diagnosed primarily because of an elevated PSA level alone, or T2 and higher tumors (N=1,923), diagnosed as a result of having a palpable tumor or evidence of non-organ confined disease. As shown in Table III, except for the SNP at 7p15 (rs10486567), no significantly different allele frequencies between these two case groups were found for the remaining 15 SNPs (nominal $P>0.05$). For example, SNP rs4430796 at 17q12 was strongly associated with PSA levels in controls ($P=7.9\times 10^{-5}$); however, the frequency of its risk allele was 0.61 for both T1c patients and T2 and higher patients. These results suggest PSA screening does not significantly inflate the frequency of alleles that are associated with PSA levels in cases.

DISCUSSION

Eeles et al. reported significant associations between several putative prostate cancer risk alleles and PSA levels in men without a prostate cancer diagnosis. The significance of this observation and its implications on the use of PSA as a screening tool has not been defined. The present study represents the first comprehensive and systematic evaluation of this association for all SNPs reported to be associated with prostate cancer risk in recent genome-wide association studies [19–26]. Results from this study revealed that six of these prostate cancer risk-associated SNPs were statistically associated with PSA levels among men without a prostate cancer diagnosis in Sweden. More prominently, these six SNPs have a strong and highly statistically significant cumulative effect on PSA levels.

The associations of PSA levels with three of the six SNPs (at 10q11, 19q13, and Xp11) in controls were consistent with the results from 1,646 control subjects from the Eeles' [26] study. The direction of this association was the same between these two studies. The associations of three other PSA associated SNPs (at 7p15, 10q26, and 17q12) reported in our study were not evaluated in the UK study [26].

Except for the SNP at Xp11, the other five PSA-associated SNPs are located in genomic regions with known genes; *JAZF1* (7p15), *MSMB* (10q11), *CTBP2* (10q26), *HNF1B* (17q12), and *KLK3* (19q13). SNP in the 3' UTR of the *KLK3* gene at 19q13 (rs2735839) is particularly relevant because this gene encodes the PSA pre-protein. Other SNPs in this gene have been previously evaluated in several study populations [3–18]. The most widely studied SNPs in this gene are located in the androgen response elements (AREs) in the 5'-upstream transcription regulatory region of the gene: rs266882 is located at position-158 bp, and rs925013 is located at position-4,643 bp with respect to the transcription start site. However, association results were inconsistent in these different study populations. In contrast, the association of PSA levels with the SNP in the 3' UTR (rs2735839) was highly significant in the UK ($P=6.1\times 10^{-8}$) and was consistently replicated in this study ($P=0.05$) and another study population from Johns Hopkins Hospital ($P=2.9\times 10^{-3}$) [29].

The cumulative effect of multiple SNPs on PSA levels may have an important clinical implication. If genetic variants and PSA are independently associated with prostate cancer risk, a genetic-specific PSA cutoff value may be used to improve the discriminatory performance of PSA by accounting for an important source of variation in PSA levels due to genetic variants. If men inherit multiple alleles that are associated with higher PSA levels, they may have PSA levels higher than that of the majority of men of their age. Consequently, applying a uniform PSA cutoff value may lead to a higher sensitivity and lower specificity in these men. On the other hand, if men inherit fewer alleles that are associated with higher PSA levels, they may have PSA levels lower than that of the majority

of men of their age. A uniform PSA cutoff value may lead to a lower sensitivity and higher specificity among these men.

There are several possible explanations for observing associations of these SNPs with both PSA levels in controls and prostate cancer risk (dual associations). First, such findings are expected if these alleles are truly associated with prostate cancer risk and reflect undetected prostate cancer cases in the control group. Under this assumption, control subjects who carried these alleles may have a higher PSA levels and more likely have undiagnosed prostate cancer. This assumption is supported by the finding from the PCPT study where ~15% of men who do not have indications for prostate cancer (PSA less than 4.0 ng/ml and normal DRE) were diagnosed with prostate cancer at an end-of-study biopsy, and the likelihood of being diagnosed with prostate cancer increased with PSA levels [2].

Second, the dual associations are also possible if these alleles are associated with higher PSA levels which in turn lead to a higher likelihood of being biopsied and subsequently diagnosed with prostate cancer (i.e., detection bias). Under this assumption, alleles associated with higher PSA levels would be inflated in cases and underrepresented in controls, particularly among controls selected for low PSA levels, regardless of whether the SNPs are truly associated with prostate cancer risk per se. Our results provided little evidence for inflated allele frequencies due to PSA detection bias in cases because the frequencies of these alleles were similar in T1c cases (cancer detected by elevated PSA levels alone) and T2 and above prostate cancer patients. However, our results indicate that some of the prostate cancer associations may result from biased allele frequencies in controls selected by virtue of having low PSA values. Low PSA control groups are commonly selected in prostate cancer case-control studies because of wide-spread use of PSA screening and because of efforts to decrease undetected prostate cancer patients among the control group. If cases are compared with these controls, false positive associations with prostate cancer risk may arise.

Finally, it is also possible that PSA itself may promote prostate cancer development as a growth factor protease or other causally related factor [30–35]. Recent data demonstrating that long-term prediction of prostate cancer up to 25 years before diagnosis of prostate cancer using PSA measured at age 44–50 years provided an important temporal relationship between PSA and prostate cancer [35]. Some effects of PSA, such as proteolysis of insulin-like growth factor binding protein-3 (IGFBP-3) and parathyroid hormone-related protein (PTHrP) are predicted to promote growth, although other effects of PSA, such as cleavage of latent transforming growth factor and fibroblast growth factor (FGF), might inhibit the growth of prostate cancer cells. Additional functional studies are warranted to address these issues.

Results from various analyses in our study may suggest three types of prostate cancer risk associated SNPs discovered from recent genome-wide analyses. The first type of SNP is less confounded by PSA levels, including the SNPs at 8q24, 17q12, and 17q24.3. The second type SNPs is considerably confounded by PSA levels, including SNPs at 19q13 and 7p15. The remaining SNPs may belong to a third type where the degree of bias due to PSA screening is uncertain. Study designs such as PCPT, where all men in the study were biopsied regardless of PSA levels, may be better suited to dissect these various scenarios.

CONCLUSION

Results from this study provided important evidence for cumulative effect of multiple SNPs on PSA levels in controls and suggest genetic-specific PSA cutoff value may be used to improve its discriminatory performance for prostate cancer. The finding of dual associations

of SNPs with PSA levels and prostate cancer risk warrants further studies because of multiple competing explanations for this observation. Understanding the tendency for some but not all prostate cancer risk SNPs to be associated with PSA levels could provide novel insight into both mechanisms of both prostate carcinogenesis as well as inherited risk for this common cancer.

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Fig. 1. Plasma PSA levels by quintiles of the number of PSA associated alleles of six PSA associated SNPs in all control subjects, as well as in younger (<65 years) or older (≥ 65 years) groups. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

TABLE I
Association of PSA Levels With 16 SNPs in Men Without a Prostate Cancer Diagnosis

Region	Position ^a	Allele (frequency)		No. of subjects by genotype				Least square mean PSA (ng/ml) ^c				P ^d
		Normal	Risk	NN ^b	NR ^b	RR ^b	NN ^b	NR ^b	RR ^b	NN ^b	RR ^b	
rs721048	2p15	63,043,382	G (0.81)	A (0.19)	1,126	528	55	1.57	1.55	1.57	1.57	0.86
rs2660753	3p12	87,193,364	C (0.92)	T (0.08)	1,430	240	10	1.57	1.49	2.37	2.37	0.78
rs9364554	6q25	160,804,075	C (0.69)	T (0.31)	807	692	170	1.62	1.51	1.63	1.63	0.50
rs10486567	7p15	27,749,803	T (0.24)	C (0.76)	103	604	103	1.25	1.47	1.67	1.67	3.0E-04
rs6465657	7q21	97,460,978	T (0.53)	C (0.47)	489	796	400	1.52	1.58	1.60	1.60	0.45
rs16901979	8q24 (2)	128,194,098	C (0.97)	A (0.03)	1,591	117	0	1.54	1.79	—	—	0.09
rs6983267	8q24 (3)	128,482,487	T (0.49)	G (0.51)	419	828	457	1.47	1.57	1.63	1.63	0.10
rs1447295	8q24(1)	128,554,220	C (0.86)	A (0.14)	1,271	392	44	1.54	1.62	1.36	1.36	0.87
rs1571801	9q33	121,506,927	G (0.72)	T (0.28)	870	708	131	1.58	1.51	1.75	1.75	0.79
rs10993994	10q11	51,219,502	C (0.61)	T (0.39)	627	810	264	1.44	1.58	1.79	1.79	1.0E-03
rs4962416	10q26	126,686,862	A (0.77)	G (0.23)	988	594	89	1.49	1.63	1.93	1.93	6.0E-03
rs10896449	11q13	68,751,243	A (0.54)	G (0.46)	477	867	342	1.57	1.52	1.72	1.72	0.24
rs4430796	17q12	33,172,153	C (0.44)	T (0.56)	316	883	509	1.5	1.53	1.75	1.75	7.9E-05
rs1859962	17q24.3	66,620,348	T (0.50)	G (0.50)	411	870	426	1.53	1.53	1.65	1.65	0.23
rs2735839	19q13	56,056,435	A (0.12)	G (0.88)	29	345	1,308	1.19	1.49	1.60	1.60	0.05
rs5945619	Xp11	51,074,708	A (0.62)	G (0.38)	1,048	0	641	1.51	—	1.65	1.65	0.05

^aBuild35.

^bNN denotes homozygous for non-risk allele; NR for heterozygous; and RR for homozygous risk allele.

^cPSA levels were log-transformed and adjusted for age and geographic region. The values presented were back-transformed.

^dTest were based on log-transformed PSA levels and assuming an additive model.

TABLE II

Multivariate Analysis of PSA Levels Among Men Without a Diagnosis of Prostate Cancer

	Region	Position ^a	Regression coefficient	P-value ^b
Age (year)			0.04	4.0E-21
Geographic region (2 vs. 1)			0.11	0.1
rs10486567	7p15	27,749,803	0.14	3.0E-04
rs10993994	10q11	51,219,502	0.12	4.0E-04
rs4962416	10q26	126,686,862	0.10	9.0E-03
rs4430796	17q12	33,172,153	0.13	8.0E-05
rs2735839	19q13	56,056,435	0.11	0.03
rs5945619	Xp11	51,074,708	0.11	0.02

^aBuild35.^bTest were based on log-transformed PSA levels and assuming an additive model for each SNP.

TABLE III

Frequencies of Risk Alleles in Case Subjects by Clinical Stage

Variable	Chr	Position ^a	Reported risk allele	P-value with PSA in controls ^a	Allele frequencies in cases			Test for difference T1c and T2/T3/T4	
					All ^b (N=2,893)	T1c (N=958)	T2/T3/T4 (N=1,731)	P-value ^c	P-value ^c
rs721048	2p15	63,043,382	A	0.86	0.20	0.20	0.20	0.54	0.54
rs2660753	3p12	87,193,364	T	0.78	0.10	0.10	0.10	0.58	0.58
rs9364554	6q25	160,804,075	T	0.330	0.33	0.33	0.34	0.58	0.58
rs10486567	7p15	27,749,803	C	3.0E-04	0.78	0.79	0.77	0.049	0.049
rs6465657	7q21	97,654,263	C	0.45	0.51	0.51	0.51	0.60	0.60
rs16901979	8q24 (2)	128,194,098	A	0.09	0.06	0.05	0.06	0.28	0.28
rs6983267	8q24 (3)	128,482,487	G	0.10	0.56	0.55	0.57	0.15	0.15
rs1447295	8q24(1)	128,554,220	A	0.87	0.17	0.16	0.17	0.30	0.30
rs1571801	9q33	121,506,927	T	0.79	0.31	0.32	0.31	0.75	0.75
rs10993994	10q11	51,219,502	T	1.0E-03	0.43	0.44	0.42	0.19	0.19
rs4962416	10q26	126,686,862	G	6.0E-03	0.24	0.24	0.25	0.82	0.82
rs10896449	11q13	68,751,243	G	0.4	0.49	0.49	0.49	0.85	0.85
rs4430796	17q12	33,172,153	T	7.9E-05	0.61	0.61	0.61	0.78	0.78
rs1859962	17q24.3	66,620,348	G	0.23	0.54	0.54	0.55	0.76	0.76
rs2735839	19q13	56,056,435	G	0.05	0.88	0.89	0.88	0.71	0.71
rs5945619	Xp11	51,074,708	G	0.05	0.42	0.42	0.43	0.77	0.77

^aBuild35.

^bA subset of cases (N=210) where clinical stage are T0, T1a, T1b, or could not be assessed.

^cP-values were based on allelic test assuming a multiplicative model.